The Nature of certain Ovum-like Bodies found in the Seminiferous Tubules.

By

F. A. E. Crew and Honor B. Fell

(From the Animal Breeding Research Department, University of Edinburgh.)

With Plates 18–23.

Introduction.

During the systematic examination of a goat with an abnormal reproductive system sent to this Department by Mr. T. H. Gillespie, Director-Secretary of the Scottish Zoological Park, certain peculiar ovum-like bodies were found within the seminiferous tubules of the ectopic testes.[1] In order to decide as to the exact nature of these, it was necessary to extend the investigation so as to include an examination of other abnormal gonads. To Mr. M. S. Pease, of the School of Agriculture, Cambridge, we are indebted for an undescended testis from a rabbit in which he had found bodies exactly similar to those found by us in the case of the goat, and also for permission to include a description of his series in this paper. An undescended testis from the human was sent to this Department by Sir Edward Sharpey Schafer, and one from a cat by Mr. A. Cameron, of the Royal (Dick) Veterinary College. Without this material our work must have remained very incomplete, and in acknowledging our debt we wish to render our grateful thanks.

We are also much indebted to the Staff of the Pathology Department of the University of Edinburgh, and to Mr. E. G. Glass for much practical assistance in the course of our study.

[1] 'Veterinary Journal', April 1922.

Methods.

With the exception of the goat the material was already fixed when received by us. The testis of the goat was fixed in Zenker's fluid, that of the rabbit in Bouin's fluid, and that of the frog and of the cat in 10 per cent. formalin.

The specimens were embedded in paraffin wax and sections were cut at 5–6 μ. We employed the following stains: eosin and Delafield's haematoxylin, Haidenhain's iron haematoxylin, Mallory's and Van Gieson's. A good method for displaying the interstitial cells was to stain with iron haematoxylin in the usual way and counter-stain with Van Gieson's.

The sections of the frog's gonad, which had been used in previous research, were stained with Delafield's haematoxylin and Bismarck brown.

The coloured drawings were made with the aid of a camera lucida. With the exception of fig. 17, for which a Koristka 1/12 oil immersion lens was employed, they were all drawn to the same scale with a Zeiss no. 7 objective.

Description of the Undescended Testis of a Goat.

The specimen was obtained from an adult goat whose sexual behaviour was sometimes of the male and at other times of the female type. The internal and external genitalia were also intersexual in character. The testes were discovered in the region of the external inguinal ring embedded in the subcutaneous tissue of the ventral body-wall and surrounded by a thick investment of fat.

General Structure of the Gonad.

Seminiferous Tubules.—The seminiferous tubules show greater degeneration than any other tissue in the gonad. Degeneration has not proceeded at a uniform rate throughout the testis, and the tubules may be described in three classes according to the stage which the atrophic changes have reached.

Class A.—Comparatively normal tubules. The cross-

section is circular or very slightly compressed. The nuclei of the peripheral cells are distinct and in some cases show a reticulate structure. Degenerative changes, however, are beginning to appear in the more central cells, which show no nuclear structure. The laminated basement membrane and its cells are normal.

Class B.—The more central epithelial cells are very degenerate. A single layer of epithelium remains adherent to the basement membrane. The cells of this layer are usually spaced at fairly regular intervals round the wall of the tubule. The cytoplasm is fragmentary and degenerate, but the nuclei are distinct. The chromatic material in the nucleus is in the form of irregular deeply-stained granules. The rest of the epithelium has become loosened and is spreading into the lumen as an irregular syncitium. The nuclei of the component cells are in many cases indistinguishable, and the cytoplasm is reduced to anastomosing threads.

Class C.—Even the single layer of cells adhering to the basement membrane is indistinguishable, and the basement membrane itself is degenerate and is breaking down altogether in places. The outlines of the tubules in cross-sections are most irregular. The lumina are filled with cell detritus which is often aggregated at one or more points into somewhat deeply-staining irregular masses. These masses frequently contain globules which adhere to one another giving the appearance of a minute drop of emulsion surrounded by cellular matter.

The most noteworthy features of the seminiferous tubules occur in Classes B and C. These are remarkable deeply-staining bodies which are often present in the lumina of the tubules. They are always closely invested by a layer of degenerate epithelial cells which might easily, at first sight, be taken for a corona radiata. A more detailed account of these structures and a discussion as to their nature will be given later.

Rete Testis.—The rete testis appears normal. The walls of the vessels are composed of a single layer of cubical epithelial cells resting on the surrounding fibrous tissue. The cell-limits

are well marked and the large oval nuclei show no indication of degeneration.

Intertubular Tissue.—Large quantities of intertubular tissue are present, the connective tissue being considerably in excess of the interstitial. The connective tissue appears normal. The interstitial cells have an oval nucleus showing a granular structure and very clear cytoplasm the periphery of which is difficult to make out. In many cases the cells appear as mere naked nuclei, some of which stain more densely than others. It would seem that the interstitial cells are either normal or in a very early stage of degeneration.

Tunica Albuginea.—The testis is invested by the usual fibrous capsule or tunica albuginea, the histology of which is normal. The only point of interest with reference to this structure is the presence in places of a layer of adipose tissue, some three cells deep, which splits the tunica albuginea into a peripheral and a central layer.

Vasa Efferentia.—These, like the rete testis, show no sign of degeneration. They are lined by normal columnar ciliated epithelial cells, the large oval nuclei of which show a somewhat reticulate structure. The bunches of cilia appear as protoplasmic tags extending into the lumen.

Epididymis.—The structure of the epididymis is in every way typical. It is composed of a layer of ciliated columnar epithelium resting on a basal layer. The basal cells contain large oval nuclei whose long axes are set transversely to the radius of the tube. Cross-sections of the epididymis differ from those of the vasa differentia in the presence of the basal layer, the relatively larger nuclei, and greater thickness of the columnar epithelium.

Blood-vessels.—Closely associated with the gonad in the region of the epididymis is a considerable venous plexus, the interstices of which are largely filled with adipose tissue. The substance of the gonad is richly supplied by a capillary system. In many instances the walls of the capillaries have ruptured owing to the degeneration of the walls of the semin-

iferous tubules and haemorrhage into the lumina of the tubules has resulted.

Account of the Ovum-like Structures in the Seminiferous Tubules and a Discussion as to their Nature.

We have already referred to the remarkable ovum-like bodies invested by epithelial cells which occur in the lumina of many of the more degenerate seminiferous tubules. Two of the more typical cases will now be described.

A. (Pl. 19, fig. 7) Two of these structures are present in the lumen of a single tubule. The outline is very sharply marked by a dark ring well seen in the lower specimen, showing that the refractive index is higher than that of the surrounding tissue. Both bodies are composed of concentric layers sharply demarcated and apparently homogeneous. In the lower specimen there are three such layers. The innermost is the most deeply stained and surrounds a paler central area. Each primary ring is subdivided into faintly marked secondary rings. Only two primary rings are visible in the second body. In both bodies the central area is paler and less homogeneous than the rings.

The investing cells are very degenerate. They are indistinctly delimited and the nuclei appear as deeply-stained structureless masses of vague outline. Syncitial strands connect the investing cells with those lining the tubule.

The epithelium of the tubule is somewhat less degenerate than is usually the case. The peripheral cells, presumably spermatogonia, display distinct though structureless nuclei. The cytoplasm, however, shows no definite outline, but coalesces in neighbouring cells and tends to spread out into the lumen. Most of the more central cells have disappeared except at those points where a syncitium connects the investing cells with the wall of the tubule. This is significant. It should also be noted that as a rule the more central the position of the cell the greater is its degeneration.

B. Only one body is present in the tubule. This specimen

is somewhat larger than either of those described above. The section was stained with Mallory's stain. The body is ovoid in shape and does not display the usual concentric structure. The main feature is the presence of two broad superimposed rings of small granules which stain a purplish blue, those of the lower ring having a more reddish tinge than those of the superficial circle. The general coloration of the body is difficult to describe accurately and a coloured drawing has therefore been made (Pl. 18, fig. 6). It will be seen that the centre has an orange tint whereas the periphery is bluish.

The investing cells are extremely degenerate. As seen in section they are reduced in places to a narrow band. The nuclei are almost indistinguishable. A single protoplasmic strand connects the investing cells with the epithelium of the tubule. The epithelial cells are reduced to a single degenerate layer adhering to the basement membrane. A comparatively large clear space intervenes between the wall of the tubule and the tissue surrounding the central body.

The distinctly female appearance of the external genitalia led us to think that possibly ovarian tissue would be found in the gonads. On first examining our preparations it seemed to us that such was the case. The spherical structures, two of which have just been described, had much the appearance of degenerate ova in typical, if degenerate, Graafian follicles. We regarded the pale somewhat granular central area so commonly present as the nucleus, the dark line bounding the body as a zona pellucida, and the investing cells as the corona radiata. The intervening spaces between the investing cells and the epithelium lining the tubule could be interpreted as cavities for the liquor folliculi, and the epithelium adhering to the basement membrane would, of course, correspond with the epithelium of the Graafian follicle. More extensive examination, however, revealed the presence of obviously homologous bodies with the concentric structures so marked as to remind the observer of a starch granule. These obviously were not of ovarian nature, as was also shown by the fact that in some instances two or more occurred in the same tubule.

As is well known, it is rare to find more than one ovum in a single Graafian follicle. It was significant also that a careful search did not reveal one of these bodies in the interstitial tissue. On considering these facts, and knowing that the seminiferous tubules were in process of atrophy, we came to the conclusion that the bodies were degeneration products. Further investigation provided what seems a series of stages in their formation. These will now be described.

(1) (Pl. 19, fig. 8) A quantity of cell detritus appears in the lumen attached by protoplasmic threads to the epithelial cells adhering to the basement membrane. No cell structure is visible. On the left of the mass is an aggregation of deeply-staining globules, each with a somewhat lighter central area. The epithelium of the wall of the tubule is very degenerate, only the nuclei of the peripheral cells being recognizable. A small group of erythrocytes is seen in the lumen, the presence of which is probably due to the rupture of a capillary in the tubule wall.

(2) (Pl. 19, fig. 9) In the upper right-hand corner of the photograph is figured a tubule containing an aggregation of cellular material which to the right forms a deeply-staining mass similar to that described above. It is composed of four large globules which appear to be coalescing. These are surrounded by detritus the cellular nature of which is still apparent. The cells lining the tubules are reduced to a narrow darkly-stained layer fused to the basement membrane.

(3) In the same illustration appears a single ovoid body in a very degenerate seminiferous tubule. The body contains a number of highly refractile granules and shows no concentric structure. It is surrounded by protoplasmic débris displaying little or no cell structure, except at one point where it is connected with the wall of the tubule. The tubule itself is of very irregular outline and the basement membrane appears to be breaking down in places.

(4) (Pl. 19, fig. 10) The tubule in this instance is less degenerate than in the cases previously described. As usual, however, only the peripheral layer of the epithelial cells is present.

These show distinct nuclei, and the outlines of the cytoplasm, though irregular, are distinguishable.

The centre of the lumen is occupied by an aggregation of cells surrounding a circular body composed of two concentric rings and a darker central area. The investing cells show two distinct layers: an outer layer in which the nuclei are visible, and an inner layer presenting no cell structure whatever. This inner layer might be regarded as part of the central body which it closely resembles both in general structure and staining reaction. Though of an asymmetrical contour which corresponds with that of the outer layer, it displays none of the angularities of the latter. The appearance suggests that during life surface tension was causing it gradually to assume a spherical shape.

(5) (Pl. 18, fig. 4) This section was stained with Mallory's stain and a coloured drawing has been made to show the somewhat remarkable staining reaction. A single circular body of rather small size is present in a seminiferous tubule. The body consists of a whitish central area surrounded by a bright blue ring. A circle of more darkly-stained granules is present. The body is surrounded by a large mass of débris which on one side spreads out into a syncitium connecting the central structure with the wall of the tubule, and on the other side is condensing into a narrower more deeply-stained layer. There is a sharp line of demarcation between the body and the surrounding protoplasm which stains an orange brown. The epithelial cells are arranged in the usual single degenerate layer applied to the basement membrane.

Discussion.—From a study of the series of specimens which have been described above it would appear that the process of formation of these remarkable bodies is somewhat as follows:

In the seminiferous tubules we have seen that degeneration spreads from the centre towards the periphery. The chief factor in this degeneration seems to be a gradual softening of the protoplasm of the epithelial cells. As this softening increases, the innermost cells can no longer adhere to the

layer beneath, but drift into the lumen of the tubule where they form the syncitial masses we have described. As more cells are added, these masses condense into more deeply-staining aggregates. The process of liquefaction continues until the more central cells lose all cytological structure and finally give rise to colloid globules which, adhering together, give the emulsoid appearance seen in (1) (Pl. 19, fig. 8) and in (2) (Pl. 19, fig. 9). If the desquamation and liquefaction of the different layers of cells are rapid, a single large colloid globule is produced, as in B (Pl. 18, fig. 6). Usually, however, the process is more gradual. Calcification appears to follow the colloid degeneration and, if desquamation and liquefaction take place slowly, may wholly or partially metamorphose the colloid globule formed by the first layer of cells, before liquefaction of the next layer is completed. In this way a body having a concentric structure is produced, each of the rings seen in the cross-section representing a layer of cells. It would appear that these objects resemble corpora amylacea in significance and in mode of formation. In (5) (Pl. 18, fig. 4) we have an example of gradual degeneration. Though the central structure is comparatively small, calcification is already complete. The preparation is stained with Mallory's stain, and the central structure has taken on the Aniline Blue, whilst the investing cells have stained with Orange G. In B (Pl. 18, fig. 6) we have, as has already been stated, an example of rapid colloid degeneration. The body which is here figured occurred in the same section as (5) (Pl. 18, fig. 4) but has stained with Orange G. like the epithelial cells of the tubule. Calcification, however, has at length set in as is shown by the blue coloration at the periphery.

On studying sections stained with Van Gieson's stain, it was found that the small globules and the most degenerate of the epithelial cells give the orange coloration characteristic of colloid degeneration, whereas the larger, concretion-like structures stain a deep magenta (Pl. 18, fig. 1). This also lends support to the view that the spherical structures have been produced by colloid degeneration followed by calcification.

Description of an Undescended Testis of a Rabbit.

The rabbit from which this testis was obtained was killed at the age of one year and seven months. The other testis was scrotal and normal. On section the testis was found to be degenerate and to contain a number of ovum-like bodies similar to those described in the goat.

General Structure of the Gonad.

Seminiferous Tubules.—The seminiferous tubules are small and degenerate.

Epithelium.—The condition of the epithelium presents considerable variation in different tubules. In some the cells are comparatively normal and completely fill the lumen. In other tubules the cell-limits are indistinguishable except in the peripheral layer, and the lumen contains a loose syncitium of protoplasmic débris (Pl. 20, fig. 11). Spermatogenesis appears to have reached the spermatocyte stage and to have stopped at that point. Most of the peripheral cells are typical spermatogonia. The more central cells are mostly large clear spermatocytes in various stages of synapsis.

As in the goat the more central nuclei on the whole display most degeneration. The nuclei of the peripheral spermatogonia are large, oval, and of a finely granular structure, whilst those lying farther in the lumen are of irregular shape, smaller size, and are more deeply staining. In many cases the epithelial cells are in active mitosis, but in the more central spermatocytes the process does not appear ever to be completed. The nucleus swells up to twice its original size and appears as a large clear vesicle containing darkly-staining chromosomes or nuclear skein. The chromatin material subsequently breaks down into smaller granules, and a further increase in the volume of the nucleus is noted. Such degenerate nuclei have been found surrounded by a mass of semi-fluid cell detritus, and in such cases would appear to serve as centres around which intratubular bodies are being formed (Pl. 20, fig. 12). Several instances of pluripolar mitosis were seen in the epithelium—a sign of degeneration.

Basement Membrane.—The basement membrane of the tubules presents a somewhat remarkable appearance (Pl. 20, fig. 13). Between the basement membrane cells and the epithelium is a layer, the substance of which is in some cases almost amorphous and in others distinctly fibrous. Elongated nuclei occur either within or central to this layer. Rudiments of this structure are present in all the tubules. In some it has become so thickened that the epithelial cells are compressed into a small mass in the centre of the tubule, and in a few instances are completely obliterated. The layer appears to be white fibrous tissue in various stages of development and with Van Gieson's and Mallory's stains takes on the same coloration as does the intertubular fibrous tissue. It is presumably formed by the cells of the basement membrane.

The same phenomenon was observed in the human testis (Pl. 20, fig. 14). In this specimen the majority of the seminiferous tubules are completely filled with a lightly-staining substance of gelatinous appearance bounded by the cells of the basement membrane. Occasionally a small central space containing cell débris remains. With Van Gieson's stain this material takes on the acid fuchsin as does the white fibrous tissue in other parts of the gonad. In some tubules fine fibres can be distinguished in the amorphous matter. A similar gelatinous layer invests groups of interstitial cells and the tubules of the rete testis. In these latter cases the substance is apparently produced by the neighbouring connective tissue cells.

It would appear that increase in fibrous tissue is correlated with colloid degeneration of the germinal epithelium. Large quantities of fibrous tissue are conspicuous both in the goat and in the rabbit, but not in the undescended testis of a cat (to be described later) in which degeneration had only just begun, nor in the testis of a pig in which spermatic tissue has undergone fatty degeneration. Our explanation of this correlation is that the colloid produced by the degeneration of the germ cells has two possible fates :

(1) It may form large colloid globules in the centre of the lumen.

(2) It may soak between the peripheral epithelial cells into the intertubular tissue and stimulate the connective-tissue cells to an increased production of white fibrous tissue. Such a condition is commonly met with in cancer and other pathological states in which colloid or mucoid degeneration is in progress. The white fibrous tissue is usually laid down around some solid structure such as a tubule or an aggregation of cells. This is the condition in the human.

The colloid may follow both courses in the same testis as in the case of the goat and the rabbit.

Stone, in his paper on a pseudo-hermaphrodite goat,[1] mentions a ' layer of hyaline material ' within the basement membrane of the tubules. Doubtless this is of the same nature as the structures we have described.

Intertubular Tissue.—As in the goat, the intertubular tissue is present in large quantities. It is composed chiefly of white fibrous tissue and interstitial cells. The bundles of fibrous tissue divide the gonad into a number of small compartments in which lie the interstitial cells.

The interstitial cells are well developed and very numerous. The cell-limits are distinct. The cytoplasm is granular and contains a large circular nucleus of finely granular structure. A nucleolus is present. The interstitial cells appear to be in rapid proliferation in certain areas in which numerous mitotic figures are seen (Pl. 21, fig. 15). In places the interstitial cells seem to be undergoing a remarkable metamorphosis (Pl. 21, fig. 16). The cytoplasm increases in volume and becomes more granular, while the nucleus takes up an eccentric position. The cytoplasm finally increases to about twice its original size, and the nucleus is situated at the extreme periphery—it may even cause a slight bulging of the cell wall. Such cells are usually formed in patches which are clearly demarcated from the surrounding tissue. They may, however, occur in small groups in which the various stages in the transformation can be followed. It would appear that the granular cells are fat-

[1] R. S. Stone, " Atypical Male Sex-ensemble in the Domestic Goat ". ' China Medical Journ.', v. 34, November 1920.

forming.[1] In the very degenerate abdominal testis of a pig which has been examined in this Department we meet with a precisely similar type of cell.[2] In this case, however, the proliferation has been completed and enormous quantities of interstitial tissue are present. The fibrous tissue has almost disappeared. All the interstitial cells have assumed the fat-forming character we have described, but are at a slightly more advanced stage than is usually the case in the rabbit in that the cytoplasm shows small circular vacuoles from which the fat has been dissolved during fixation. In the human undescended testis to which we have already referred a similar condition is found. The immense increase in interstitial tissue, so striking a feature in the pig and rabbit, was not seen, but all the interstitial cells have taken on the character of fat-forming cells. The cytoplasmic vacuoles are much larger than in the pig, and in some cases occupy most of the cell.

Tunica Albuginea.—The tunica albuginea is considerably thickened at one point but otherwise appears normal.

Epididymis.—The epididymis (Pl. 21, fig. 17) is closely invested at all points by a thick layer of adipose tissue which separates it from the gonad. The cytology of the epithelial cells appears almost normal, although in certain of the cells the cytoplasm has a slightly vacuolated appearance. Cilia are visible. The lumen is filled with a lightly-staining coagulum in which appear circular vacuoles. Deeply-staining cell detritus is also present.

Intratubular Bodies.—Ovum-like bodies are present in large numbers. They closely resemble those found in the goat but have not reached such an advanced stage of formation.

Structure.—Calcification appears in comparatively few; most give the vivid orange coloration with Van Gieson's stain, characteristic of colloid degeneration (Pl. 18, fig. 3). When

[1] Since writing the above this question has been investigated further. It would appear that the cells are not 'fat-forming' but are enlarged owing to accumulation in the cytoplasm of various nutritive materials. A paper dealing fully with this subject is in the press.

[2] 'Veterinary Journal', March 1922.

stained with Mallory's stain they are affected by Orange G. only, whatever the period of staining. A few display the blue centre and bluish superficial film which we take to represent incipient calcification. Pairs of serial sections stained alternately with Mallory's and Van Gieson's stains show that those parts of a body which stain blue with Mallory's stain, stain magenta or remain colourless with Van Gieson's stain. In many of the tubules the bodies are surrounded by an aggregation of cell material, but in others they lie free in the lumen. The concentric structure characteristic of the bodies described in the goat is most marked but the number of rings is smaller (Pl. 21, fig. 18). Indeed, in most cases only one ring is present surrounding a more lightly-staining somewhat granular central area. The primary ring is usually marked by secondary rings. Sometimes two or more primary rings occur.

Formation.—The method of formation of the intratubular bodies appears to be the same as in the goat, viz. by the liquefaction of aggregates of epithelial cells. This liquefaction may take place round a degenerate epithelial nucleus, as described above (Pl. 20, fig. 12), or may proceed directly with the formation of one or more colloid globules surrounded by a mass of desquamated cells. The centre is formed by a degenerate nucleus, the ovum-like appearance is very striking. As has already been stated, calcification has begun in relatively few instances.

Description of the Undescended Testis of a Cat.

The testis was taken from a six months old animal and was found at the external aperture of the inguinal canal. The other testis had been removed from the scrotum a month previously and there was then no sign of a second testis. The cat was otherwise normal.

A microscopical examination reveals arrested development and early degenerative changes.

Seminiferous Tubules.—The seminiferous tubules are of normal size and shape but show only the first stage of sperma-

togenesis. Mitotic figures are rare. The lumina are occupied by a protoplasmic syncitium. The nuclei of the epithelial cells are mostly arranged in a single peripheral layer, although many lie more centrally. In the majority of cells the outline of the cytoplasm is indistinguishable as these cells tend to coalesce laterally. Others, however, are clearly defined.

The epithelial cells appear to be of three types as follows :

(1) Typical spermatogonia. These contain a reticulate nucleus with well-marked nucleolus. The limits of the cytoplasm in neighbouring cells cannot be made out.

(2) Large cells lying immediately central to the basal layer. They may be twice or three times as large as the basal cells. The cytoplasm is very hyaline and the cells in consequence are sharply demarcated from the more darkly-staining surrounding tissue. The nucleus varies in size and staining capacity. Typically it is finely reticulate and contains a nucleolus. It may, however, be small and dense, or so large as to fill two-thirds of the cell. In the latter case the chromatin material is reduced to a few small strands and granules surrounding a very large nucleolus (Pl. 18, fig. 2).

These large clear cells are probably late spermatogonia. Many appear degenerate, and in a few the nucleus is altogether disintegrating. It is noticeable that the larger the cell the greater is the relative size of the nucleolus. This fact will be referred to later.

(3) The third type of cell has a rather small granular ovoid nucleus containing a nucleolus. The cytoplasm is pyramidal in form. It is attached at the base to the basement membrane and is often drawn out into a protoplasmic filament. These we regard as cells of Sertoli. The lumina of the tubules often contain a deeply-staining amorphous substance which appears to be a mixture of protoplasmic detritus and coagulated fluid. Degenerate nuclei are usually present in such masses (Pl. 22, fig. 19). Small deeply-staining globular bodies (Pl. 22, fig. 20) occasionally occur in the central syncitium of the tubules and more rarely among the peripheral cells. They are found to stain brightly with the Orange G. of Mallory's stain. It would

appear that they are colloid globules of the same nature as those described in the goat and the rabbit. A coloured drawing has been made (Pl. 18, fig. 5) from one of the more typical of these. The figure was taken from a section stained with Mallory's stain. The body is seen to consist of a darker central area surrounded by a lighter ring. It is invested by a protoplasmic syncitium. The tubule is lined with epithelial cells most of which are attached to the basement membrane.

The formation of the colloid body seems to be initiated by the nucleolus of one of the more centrally situated nuclei. The process appears to be as follows : The nucleolus increases to several times its original size, apparently absorbing the rest of the nuclear material. The nucleus becomes more and more vacuolated and is finally reduced to a large clear vesicle containing an enormous nucleolus. All stages intermediate between a normal nucleolus in a reticulated nucleus and a well-developed colloid globule are seen (Pl. 18, fig. 2).

Intertubular Tissue (Pl. 22, fig. 21).—The Interstitial Cells show reduction instead of hyperplasia. They occur in columns and groups which are usually closely applied to the basement membrane of the tubule.

A large elongate group is found intercalated between two layers of the tunica albuginea. The nuclei are spherical and have a very distinct nucleolus. They are usually eccentrically situated. The cytoplasm is very vacuolated, especially in those cells which occur in the tunica albuginea. In many of the latter the cell is almost an empty vesicle. Fibrous tissue is present in small quantities.

Epididymis.—The epididymis appears normal and typical. It is lined with the usual columnar ciliated epithelium resting on a basal layer. The lumen in places contains masses of débris. The connective-tissue stroma is normal.

Vasa Efferentia.—These consist of the usual layer of columnar ciliated cells somewhat shallower than that of the epididymis.

Tunica albuginea.—The tunica albuginea is typical and normal.

Description of the Displaced Testis of a Frog.

A frog, Rana temporaria, was killed in April 1916. It was an adult, measuring 5·2 cm. from the tip of its snout to the symphysis of the pubis, while its accessory sexual apparatus and its secondary sexual characters were entirely and typically male. During the dissection it was noticed that while the left gonad was a testis, normal in every respect, the right gonad was missing. But there was a tumour 10 mm. long and 6 mm. broad, encased in a fibrous tissue capsule and firmly attached to the muscle-sheaths of the m. rectus abdominis and m. triceps femoris and lying upon and distal to the right groin. Naturally it was assumed that this tumour was the missing testis and search was made for vasa efferentia but none could be found, for the tumour was not connected with any part of the genital system. Further dissection showed that there was a hernia, the sac of which was provided by the abdominal peritoneum and which was covered by the stretched fibres of the m. obliquus externus. The neck of the sac was almost completely obliterated by fibrous over-growth and was adherent to the adjacent surfaces of the m. rectus abdominis and m. triceps femoris at the site of their origin, while the dorsal surface of the tumour was firmly adherent to the ventral surface of the latter muscle. It is of interest to note that the location of this tumour is very nearly that of a testis in the scrotum: it lay close to where the external ring of the inguinal canal would be.

On section the left gonad has the structure of the normal testis. The tumour proved to be the missing testis, but its structure is peculiar in that while the tissues near the periphery have the normal structure of a testis, the central parts of the gonad consists of a loose matrix of indefinite tissue amongst which many large bodies closely resembling ova are found. The gonad has the structure of the intermediate gonad as defined by Schmitt-Marcel [1] and of the testis of indirect development as described by Witschi.[2] The testis is encased

[1] 'Arch. f. mikr. Anat.', vol. 72, p. 516, 1908. [2] Ibid., vol. 85, 1914.

in a thick capsule of fibrous tissue and muscle-fibres and branches of the femoral artery provide a blood-supply by which the gonad is nourished. No vasa efferentia are present.

The condition of the seminiferous tubules shows that there had been active spermatogenesis prior to the displacement of the gonad. Many of the tubules are still comparatively normal and are thickly lined with spermatozoa which are arranged with the heads attached to the basement membrane and the tails extending into the lumen (Pl. 23, fig. 22). In other tubules, however, we find evidence of degeneration. As in the cases of goat, rabbit, and cat, degeneration takes place from within outwards. The tails lose their outline and fuse to form a lightly-staining granular mass in the centre of the tubule. The heads may remain distinct, but in more advanced stages of degeneration they also tend to coalesce.

As we have already stated, large ovum-like structures completely filling the lumen are present in some of the tubules (Pl. 23, fig. 23). These consist of a deeply-staining peripheral layer which is sharply demarcated from a more lightly-staining somewhat granular central area. So far as we can judge from the limited number of serial sections at our disposal, it seems probable that the bodies are tubular and follow the convolution of the tubule in which they are contained. We are, however, not certain on this point, although it is significant that the bodies almost invariably occur in groups of two or three, as if several convolutions of a single tubule had been sectioned at once.

We regard these structures not as ovarian in character but as a result of a more advanced stage in the degeneration of the seminiferous tubules. It would appear that they represent an early phase of that colloid degeneration which has been described in the goat, rabbit, and cat. Actual colloid has not yet been formed, but liquefaction has advanced so far that the heads and tails have coalesced into single protoplasmic masses. The dark peripheral layer appears to be formed by the heads, the light central area by the tail, and the seminal fluid occluded in the tubule.

Summary.

In the study of the ectopic testes of a goat, rabbit, and frog, bodies were found bearing a strong resemblance to ova within Graafian follicles. However, these proved to be not ovarian but the degeneration products of the germinal epithelium of the seminiferous tubules. The bodies appear to be produced by the gradual liquefaction of masses of desquamated cells, whereby large colloid globules are formed which subsequently undergo calcification. The gonads of the frog, rabbit, and goat present a progressive series of these degenerative changes.

1. In the frog the bodies are still protoplasmic and are formed by the liquefaction of spermatozoa. The tails of the sperms give rise to a central lightly-staining area which is surrounded by a broad dark ring formed by the lateral coalescence of the heads.

2. In the rabbit degeneration has in most cases reached the colloid stage. Large spherical bodies, which with Van Gieson's stain give the orange coloration characteristic of colloid, are found in the lumina of many of the seminiferous tubules. A few are showing signs of calcification. They are formed (*a*) by the liquefaction of protoplasmic detritus around the nucleus of a degenerate spermatocyte; (*b*) by the coalescence of several small colloid globules formed in the centre of a mass of cell débris.

3. In the goat the large colloid globules are undergoing calcification, as is shown by a study of sections stained with Mallory's and Van Gieson's stains. Only a few of the smaller globules give the colloid coloration. The formation of the bodies appears to be the same as in the gonad of the rabbit.

The undescended testis of a young cat was also examined microscopically. This shows a very early stage of degeneration. Spermatogenesis has ceased and the syncitium in some of the tubules is degenerate. Minute colloid globules which seem to be derived from the hypertrophied nucleoli of spermatocytes occur in the syncitial protoplasm of some of the tubules.

Points of interest in connexion with this study are as follows :

1. It seems probable that many of the structures in abnormal testes which have been regarded as ova are in reality of a degenerative character, similar to that of the bodies we have described.

2. The interstitial cells of the rabbit's testis are in certain areas being metamorphosed into what appear to be fat-forming cells.

3. The colloid degeneration of the spermatic epithelium is correlated with an increased production of white fibrous tissue. This phenomenon may be explained on the assumption that some, or, as in the human, the whole of the colloid percolates through the peripheral layer of cells and stimulates the cells of the basement membrane and of intertubular connective tissue to greater activity.

EXPLANATION OF PLATES 18–23.

Plate 18.

Fig. 1.—Goat. Seminiferous tubule. ×470. Stained with Van Gieson. All the epithelium is desquamated into the lumen to form a syncitial mass in which several ovum-like bodies are in the process of formation. Two complete bodies are seen, the lower one is completely calcified but the centre of the upper one still gives the orange coloration characteristic of colloid. A number of colloid globules are seen in other parts of the lumen.

Fig. 2.—Cat. Spermatocytes within seminiferous tubules. ×900. Stained Mallory. Shows every stage in the formation of colloid globules by hypertrophy of the nucleolus. In (*a*) and (*c*) the cells are centrally situated, in (*b*) they are peripheral.

Fig. 3.—Rabbit. Seminiferous tubule. ×470. Stained Van Gieson. Two ovum-like bodies are present surrounded by desquamated cells. They show the vivid orange coloration characteristic of colloid. Calcification has not yet set in. The thickened basement membrane is also seen.

Fig. 4.—Goat. Seminiferous tubule. ×470. Stained Mallory. A single circular body lies within the tubule and consists of a central whitish area surrounded by a bright blue ring. A circle of more darkly-staining granules is also present and the body is invested by a large mass of débris which at one side spreads out into a syncitium connecting the central body with the wall of the tubule.

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